

β_2 adrenoceptor Arg16Gly polymorphism, airway responsiveness, lung function and asthma in infants and children

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Summary

Background We have previously reported a relationship between increased airway responsiveness (AR) in infancy and reduced childhood lung function.

Objective The current study aimed to determine whether the Arg16Gly polymorphism of the β_2 adrenoceptor (β_2 AR) gene was important to this relationship.

Methods A cohort that initially numbered 253 individuals underwent assessments of AR and lung function aged 1 month, 6 and 11 years; genotyping for polymorphisms of the β_2 AR was performed.

Results At 1 month of age, the genotype homozygous Arg16 ($n = 24$) was associated with a mean increase in log dose–response slope (AR) of 0.27 [95% confidence interval (CI) 0.07, 0.49] compared with the genotype homozygous Gly16 ($n = 58$), $P = 0.01$. At 11 years of age, the genotype homozygous Arg16 ($n = 35$) was associated with a mean reduction in the percentage of forced expiratory volume in 1 s of 5.3% [95% CI 0.3, 10.2] compared with the genotype homozygous Gly16 ($n = 65$), $P = 0.03$. There was no association between the Arg16Gly polymorphism and atopy or diagnosed asthma. However, nine of 69 individuals with the genotype homozygous Gly16 were admitted to hospital with asthma compared with five out of 111 individuals with the remaining genotypes ($P < 0.05$).

Conclusion The Arg16Gly polymorphism may be important to the association between increased AR in infancy and reduced lung function in childhood and may also be a determinant of asthma severity in children but not asthma *per se*.

Keywords bronchial hyperreactivity, genetics, infant, longitudinal study, respiratory function tests

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Introduction

We have previously described a relationship between increased airway responsiveness (AR) at 1 month of age and reduced lung function in later childhood in a population unselected for asthma [1, 2]. In a preliminary report, this observation has been confirmed elsewhere [3]. The very early onset of this relationship suggested that an underlying factor acts before or very shortly after birth and a genetic factor affecting airway tone or response would be consistent with this observation. The β_2 adrenoceptor (β_2 AR) is a transmembranous protein expressed by airway smooth muscle cells [4]. Stimulation of the β_2 AR results in cAMP-mediated muscular relaxation [4] that is important to the regulation of airway tone, which, in turn, is also important to AR and lung function. Polymorphisms of the β_2 AR might be associated with either increased AR or reduced lung function, and could be a plausible mechanism for the relationship observed by our group.

There are two common amino acid substitutions in the β_2 AR, these are at the 16th (Arg $\rightarrow$ Gly) and 27th (Gln $\rightarrow$ Glu)

positions [5]. These alleles are in strong linkage disequilibrium; most chromosomes with the Arg16 allele also carry the Gln27 allele and the Arg16Glu27 haplotype is very uncommon. Several studies have examined relationships between polymorphisms of the β_2 AR, AR and lung function [6–12]. Although some of these results are conflicting, they do suggest that the β_2 AR gene has an important role in determining the level of AR and lung function in many different populations.

Our longitudinal study of AR and lung function from 1 month old has been completed at 11 years of age and the genotype of most individuals has been determined. This population is a unique resource as it is the only population that has had AR and lung function measured soon after birth with a comprehensive longitudinal follow-up. In view of the previous findings [1, 2, 12], we hypothesized that polymorphisms of the β_2 AR would be associated with increased AR in infancy and reduced lung function in childhood.

Methods

Subjects

Subjects were participants in a prospective study of lung function, AR and atopy beginning in infancy and were

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enrolled from an unselected, Caucasian population. Parents attending an ante-natal clinic enrolled their child into the study before birth but infants subsequently born prematurely or with neonatal respiratory illness were excluded. At enrollment, a parental history of asthma and/or smoking was recorded. A detailed description of enrolment is also given elsewhere [13]. This study was approved by the Medical Ethics Committee of Princess Margaret Hospital and the Confidentiality into Health Information Committee. Written consent was obtained from parents and verbal assent from children.

Assessment aged 1 month

The techniques used have been described [13]. Infants were induced to sleep with chloral hydrate and maximal flow at functional residual capacity ($V'_{\max FRC}$) was determined from the rapid thoraco-abdominal compression technique during tidal breathing. Doubling concentrations of nebulized histamine were then administered (0.125–8 mg/mL) until there was either a 40% reduction in $V'_{\max FRC}$ or the final concentration had been administered. AR was expressed as both the provocative concentration causing a 40% reduction in $V'_{\max FRC}$ (PC_{40}) or the dose–response slope (DRS). The DRS was calculated by dividing the percentage reduction in $V'_{\max FRC}$ by the concentration of histamine last administered. As previously [1], those individuals where $V'_{\max FRC}$ did not fall by 40% after inhalation of the maximal concentration of histamine were assigned the value $PC_{40} = 16 \text{ mg/mL}$.

Assessment at 6 and 11 years

Parents assisted children to complete a modified respiratory questionnaire [14]. A portable spirometer (Pneumochek Spirometer 6100; Welch-Allyn, Skaneateles Falls, NY, USA) was used to measure forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), forced expiratory flow (FEF_{25-75}) and peak expiratory flow (PEF) in accordance with published guidelines [15]. The rapid inhalation technique [16] was used to determine AR to inhaled histamine, and AR was expressed as a DRS that was calculated as the percentage (%) of reduction in FEV_1 per micromole of histamine inhaled [17]. Bronchodilator therapy was withheld for at least 6 hours prior to testing. Skin reactivity to cow's milk, egg white, rye grass, mixed grass, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, cat dander, dog dander, *Alternaria alternans* and *Aspergillus fumigatus* (Hollister-Stier, Elkhart, IN, USA) was assessed by skin prick testing. The positive control was histamine sulphate (10 mg/mL) and the negative control was 0.9% saline. A positive skin test was defined as a weal at least 3 mm in its longest dimension.

Screening for polymorphisms

Blood was taken for DNA extraction on the occasion of the assessment either at 6 or 11 years of age. Polymorphisms at the 16th and 27th position of the β_2AR were screened for using techniques described in detail previously [6, 18]. Briefly, using primers designed with reference to the published sequence of the β_2AR gene, a fragment spanning the polymorphism of interest was amplified by PCR. Genotyping

was determined at the time of the 6-year follow-up using allele-specific oligonucleotide hybridization [6], for the 11-year-study restriction enzyme length polymorphism analysis was used [18]. Genotyping was confirmed in a random 10% of samples by direct sequencing.

Statistical analysis

The distributions of PC_{40} and DRS were skewed with long right-hand tails and were log transformed prior to analysis (a constant of three was added to DRS to allow values of zero and less to be included in the analysis). The PC_{40} and DRS were expressed as geometric means (GMs) and 95% confidence intervals (CIs) ($1.96 \times \text{SEM}$). $V'_{\max FRC}$ was expressed as % of predicted after adjustment for weight, length, age, gender and maternal smoking in pregnancy [19]. Individuals admitted to hospital with a primary diagnosis of asthma were identified from the Maternal and Child Health Database of Western Australia, which records details of admissions to all hospitals in the state with the exception of individuals discharged from emergency departments.

The study had power at the 80% level (assuming significance at 5% and knowing the allele frequencies and variable descriptive statistics for the study subjects) to detect a 0.3 difference in log DRS at 1 month of age and a 6.5% difference in FEV_1 at 11 years of age between groups homozygous for Arg16 and Gly16.

General linear models were constructed to permit % of predicted lung function at 6 and 11 years age to be expressed adjusting for height, gender, and parental histories of smoking or asthma. Student's *t*-test, Mann–Whitney *U*-test and analysis of variance were used as appropriate to compare differences in % lung function for infants and children and AR between groups of infants determined by genotype. General linear models were created to determine the relationship between DRS in children and genotype adjusting for atopy. Significance was assumed at the 5% level. Analyses were performed using a standard statistical software package (SPSS release 9.0.1, SPSS, Chicago, IL, USA).

Results

Characteristics of the population

Figure 1 displays the number of individuals followed-up at 1 month, 6 and 11 years of age. At 1 month of age, 243 infants were assessed and AR was determined in 203 infants; of these 203 individuals, 143 (71%) had genotype ascertained in childhood. At 6 years of age, 123 children were assessed of whom the genotype was known in 118 (96%). At 11 years of age, 194 children were assessed and the genotype was known in 172 (89%). Five children were assessed at 6 but not 11 years of age. Table 1 compares the original cohort with individuals seen at 1 month, 6 and 11 years where genotype was also known. Table 2 compares individuals who were assessed in whom the genotype was and was not determined.

Genotype frequencies

Genotyping was performed in 180 (71%) of the original cohort; genotype frequencies at the 16th position were: Arg/Arg

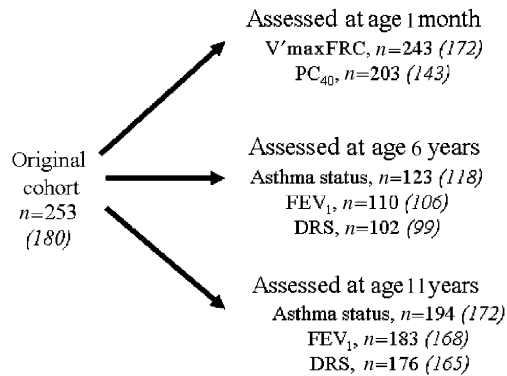


Fig. 1. A flow chart demonstrating the number of individuals assessed at ages 1 month, 6 and 11 years. Numbers in italics correspond with the number of individuals where genotype was determined. DRS, dose response slope.

20%; Arg/Gly 42%; Gly/Gly 38%; and at the 27th position Gln/Gln 32%; Gln/Glu 47%; Glu/Glu 21%. The allele frequencies at the 16th and 27th position were in Hardy–Weinberg equilibrium. There were 21 sibling-pairs involved in the study who were also genotyped, but this did not significantly alter the allele frequency.

Lung function at 1 month of age and polymorphisms of the β_2 AR

At 1 month of age, the DRS was steeper (i.e. increased AR) in individuals homozygous for Arg16 (GM 66.1 [95% CI 47.3, 98.8], $n = 24$) compared with heterozygous genotype Arg16-Gly (GM 43.8 [31.5, 60.5], $n = 62$) and the genotype homozygous Gly16 (GM 33.5 [95% CI 25.0, 41.6], $n = 58$) Kruskal–Wallis, $P = 0.03$ (Table 2 and Fig. 2). The log DRS for individuals, the genotype homozygous Arg16, was increased by a mean of 0.27 [95% CI 0.07, 0.49] compared with the genotype homozygous Gly16, $P = 0.01$. At 1 month of age, there was a trend for reduced PC₄₀ (i.e. increased AR) to be associated with the Arg16 allele: the GM PC₄₀ for infants with the genotype homozygous Arg16 was 0.74 [95% CI 0.49, 1.13] ($n = 24$), for heterozygous genotype Arg16Gly was 0.90 [95% CI 0.65, 1.24] ($n = 62$) and for the genotype homozygous Gly16 was 1.13 [95% CI 0.83, 1.53] ($n = 58$) ($P = 0.07$ between the genotype homozygous Gly16 and others); see Table 2. There was a trend for reduced % V_{max}FRC to be associated with the Arg16 allele: the mean % V_{max}FRC for infants with the genotype homozygous

Arg16 was 85% [95% CI 71, 99] ($n = 34$), for heterozygous genotype Arg16Gly was 107% [95% CI 94, 119] ($n = 70$) and for the genotype homozygous Gly16 was 98% [95% CI 87, 109] ($n = 68$) (Table 2). There was no relationship between the Gln27Glu polymorphism and DRS, PC₄₀ or % V_{max}FRC at 1 month of age.

Polymorphisms of the β_2 AR and lung function, atopy and asthma aged 6 years

At 6 years of age, there was a trend for reduced % FEV₁ to be associated with the Arg16 allele: the mean % FEV₁ for children with the genotype homozygous Arg16 was 95% [95% CI 90, 100] ($n = 26$), for heterozygous genotype Arg16Gly was 102% [95% CI 97, 107] ($n = 39$) and for the genotype homozygous Gly16 was 101% [95% CI 95, 106] ($n = 41$) (Table 2). Univariate analysis of the DRS at 6 years of age demonstrated increased DRS in individuals homozygous for Arg16 (GM 7.4 [95% CI 4.1, 12.1], $n = 24$) compared with individuals homozygous for Gly16 (GM 4.5 [95% CI 2.3, 7.7], $n = 38$), $P = 0.03$. After adjustment for atopy, the relationship between DRS and the Arg16Gly polymorphism was no longer significant. Polymorphisms of the β_2 AR at the 16th and 27th position were not related to % FVC, % FEF_{25–75}, % PEF, atopy or diagnosed asthma (Table 2).

Polymorphisms of the β_2 AR and lung function, atopy and asthma aged 11 years

At 11 years of age, the mean % FEV₁ for individuals with the genotype homozygous Arg16 was 97% [95% CI 93, 101] ($n = 35$), for heterozygous genotype Arg16Gly was 98% [95% CI 96, 100] ($n = 68$) and for the genotype homozygous Gly16 was 102% [95% CI 99, 105] ($n = 65$) (ANOVA, $P = 0.03$). The genotype homozygous Arg16 was associated with a mean reduction in % FEV₁ of 5.3% [95% CI 0.3, 10.2] compared with the genotype homozygous Gly16, $P = 0.03$. The mean % FVC [95% CI] at 11 years of age were as follows: the genotype homozygous Arg16 98 [94, 102] ($n = 35$); heterozygous genotype Arg16Gly 99 [95, 101] ($n = 68$); the genotype homozygous Gly16 103 [100, 106] ($n = 65$), ANOVA, $P = 0.03$. The genotype homozygous Arg16 was associated with a mean reduction in % FVC of 5.8% [95% CI 0.7, 10.9] compared with the genotype homozygous Gly16, $P = 0.03$. At 11 years of age, there was no relationship between β_2 AR polymorphisms at the 16th and 27th positions and the DRS, % FEF_{25–75},

Table 1. Comparison of whole cohort with individuals seen at 1 month, 6 and 11 years of age where genotype was known, n = number of individuals

	Whole cohort ($n = 253$)	Individuals where AR at 1 month and genotype were determined ($n = 143$)	Individuals assessed at 6 years of age and genotype known ($n = 120$)	Individuals assessed at 11 years and genotype known ($n = 172$)
Male (number)	56% (142)	59% (84)	53% (64)	57% (97)
History of parental asthma* (number)	31% (75)	24% (43)	28% (33)	30% (52)
History of maternal smoking during pregnancy (number)	27% (68)	24% (34)	18%† (22)	23%† (39)
DRS at 1 month‡	40.8 [37.3, 48.5]	42.3 [34.6, 51.5]	31.2 [27.8, 46.8]	41.7 [33.9, 51.0]

*Data not available for 11 fathers. † $P < 0.05$ compared with whole cohort. ‡Expressed as geometric mean [95%CI] ($1.96 \times \text{SEM}$). AR, airway responsiveness; DRS, dose–response slope.

Table 2. Details of individuals grouped by Arg16Gly genotype

	Arg/Arg (<i>n</i> = 36)	Arg/Gly (<i>n</i> = 75)	Gly/Gly (<i>n</i> = 69)	Assessed but no genotype
Male gender (%) (<i>n</i>)	44 (16)	59 (44)	62 (43)	53 (39/73)
Maternal smoking during pregnancy (%) (<i>n</i>)	19 (7)	24 (18)	26 (18)	34 (25/72)
% V'maxFRC aged 1 month [95% CI] (<i>n</i>)	85 [71, 99] (34)	107 [94, 119] (70)	98 [87, 109] (68)	99 [91, 107] (69)
Geometric mean PC ₄₀ aged 1 month [95% CI]* (<i>n</i>)	0.74 [0.49, 1.13] (24)	0.90 [0.65, 1.24] (62)	1.13 [0.83, 1.53] (58)	0.98 [0.68, 1.41] (59)
Geometric mean DRS aged 1 month [95% CI]* (<i>n</i>)†	66.1 [47.3, 98.8] (24)	43.8 [31.5, 60.5] (62)	33.5 [25.0, 41.6] (58)	38.5 [27.0, 54.4] (59)
% FEV ₁ aged 6 years [95% CI] (<i>n</i>)	95 [90, 100] (26)	102 [97, 107] (39)	101 [95, 106] (41)	103 [90, 116] (4)
Geometric mean DRS aged 6 years [95% CI]* (<i>n</i>)‡	7.4 [4.1, 12.1] (24)	5.0 [2.8, 8.1] (37)	4.5 [2.3, 7.7] (38)	3.1 [2.9, 3.4] (3)
Asthma aged 6 years (%) (ratio)§	22 (6/27)	20 (9/45)	28 (13/46)	40 (2/5)
Atopy aged 6 years (%) (ratio)§	26 (7/27)	38 (15/39)	39 (16/41)	20 (1/5)
% FEV ₁ aged 11 years [95% CI] (<i>n</i>)¶	97 [93, 101] (35)	98 [96, 100] (68)	102 [99, 105] (65)	96 [90, 102] (15)
Geometric mean DRS aged 11 years [95% CI]* (<i>n</i>)	2.4 [1.7, 3.2] (35)	2.3 [1.5, 3.4] (65)	2.4 [1.5, 3.3] (65)	2.3 [1.3, 3.7] (11)
Asthma aged 11 years (%) (ratio)§	22 (8/36)	11 (8/70)	17 (11/66)	9 (2/22)
Atopy aged 11 years (%) (ratio)§	44 (16/36)	55 (38/69)	53 (35/66)	36 (4/11)

*PC₄₀ and dose-response slope (DRS) expressed as geometric mean and 95%CI (1.96 × SEM). †*P* = 0.03 for Kruskal–Wallis test. ‡*P* = 0.03 comparing ArgArg with GlyGly. §Ratio of number affected/ total number with Arg16Gly genotype. ¶ANOVA, *P* = 0.03. There were no differences between individuals assessed and genotyped compared with those assessed but not genotyped.

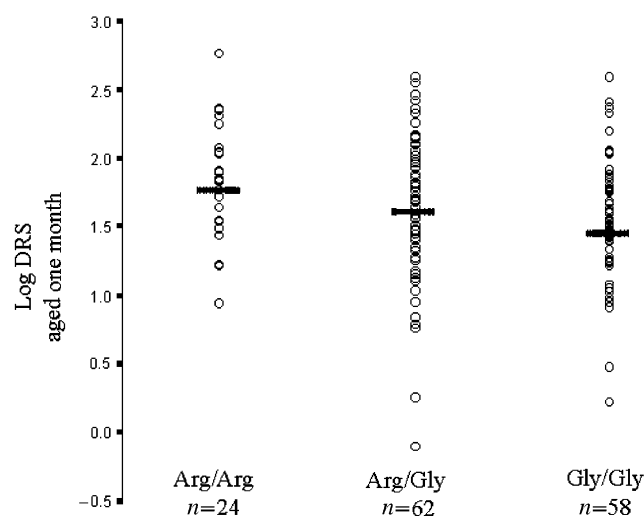


Fig. 2. Scatter plot showing values of dose response slope (DRS) at 1 month of age for groups determined by the Arg16Gly polymorphism. Horizontal lines indicate mean values. Kruskal–Wallis test, *P* = 0.03.

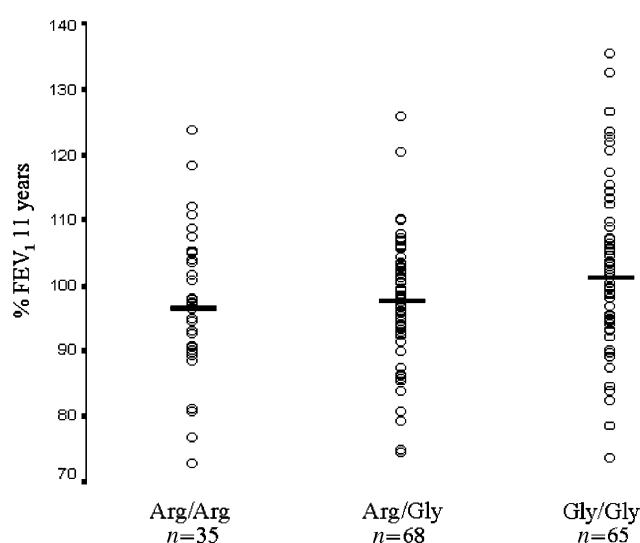


Fig. 3. Scatter plot showing values of % of predicted forced expiratory volume in 1 s (FEV₁) at 11 years of age for groups determined by the Arg16 Gly polymorphism. Horizontal lines indicate mean values. ANOVA across groups, *P* = 0.03.

% PEF, atopy or diagnosed asthma (Table 2). The relationship between reduced lung function and homozygosity for Arg16 was maintained when individuals with asthma were excluded from the analysis.

Polymorphisms of the β_2 AR and asthma admissions

Seventeen individuals were admitted to hospital with acute asthma and the genotype was determined in 14 cases. The median age at first hospitalization was 1.8 years (range 1.2–8.6 years); the median stay in hospital was 1 day (range 1–4 days). The median number of hospitalizations was two; six children were admitted on one occasion, eight children on two occasions, and one child on either four, five or six occasions. Nine individuals admitted for asthma had the genotype homozygous Gly16, four had heterozygous genotype Arg16Gly and one had the genotype homozygous Arg16

(Table 3). The odds ratio for an individual with the genotype homozygous Gly16 being admitted for asthma was 3.2 [95% CI 1.0, 9.9], *P* < 0.05 compared with other genotypes.

Discussion

This study was designed to determine whether expression of the β_2 AR Arg16Gly polymorphism was associated with the relationship between increased AR in early life and reduced lung function later in childhood [1, 2]. During infancy and childhood, the Arg16 allele was associated with increased AR and reduced lung function. The evidence therefore suggested that sequence variations of the β_2 AR could be important in the relationship between AR in infancy and lung function in later childhood. The relationships between the Arg16Gly

Table 3. Distribution of Arg16Gly β_2 adrenoceptor polymorphism among those hospitalized and not hospitalized for acute childhood asthma

	Arg/Arg	Arg/Gly	Gly/Gly*
Hospitalized	1	4	9
Not hospitalized	35	71	60

* $P < 0.05$ comparing proportion of individuals homozygous for Gly16 and hospitalized for asthma with other individuals hospitalized.

polymorphism and lung function at 6 years of age did not reach significance; however, measurements of lung function are highly variable in young individuals and we believe that with greater numbers of subjects, significance would have been achieved. The data also suggested that β_2 AR variation at the 16th position might be related to asthma severity. There was no relationship between the β_2 AR Arg16Gly polymorphism and atopy or asthma *per se* suggesting that the polymorphism may exert its effect on lung function, AR and asthma severity via an alternative mechanism.

Aspects of the results were consistent with the findings of several other studies [6, 7, 11, 12]. Increased bronchodilator response in children has been reported in Arg16 homozygotes compared with Gly16 homozygotes [11] and reduced AR has been noted in adults and children homozygous for Glu27 compared with Gln27 homozygotes [6, 7]. Summerhill et al. [12] described a 5% reduction in FEV₁ associated with Arg16 homozygosity and these data are comparable with the results at 6 and 11 years of age in this study. The results were not consistent with those of Fowler et al. [10] who reported an association between reduced AR and Arg16 homozygosity among an asthmatic population. One paediatric study has reported an association between the β_2 AR polymorphism at the 27th position and diagnosed asthma [20], but we were not able to confirm this. The reasons for the observed differences in results between studies of β_2 AR genotype is unclear, but there were a number of explanations. Genetic heterogeneity between populations may produce inconsistencies between studies [21] and environmental differences may also alter gene expression [22]. The association between the polymorphism of the β_2 AR and respiratory symptoms may have been influenced by altered lung function. Another possible explanation is that this and other studies have only considered the relationship between genotype and not haplotype on phenotype, and there are data to suggest that functionality of the β_2 AR is related to a combination of polymorphisms rather than a single polymorphism [23].

An association between the β_2 AR Arg16Gly polymorphism and increased asthma severity has not been previously described in children, but the association has been sought in adults and identified in one study [18] but not in a second study [24]. Severe asthma is usually associated with reduced lung function and increased AR and this appears inconsistent with the results of this and other studies [6, 7, 11, 12] that have associated the Gly16 allele with increased lung function and reduced AR. A possible explanation is that the increased AR conferred by the Arg16 allele permits bronchodilation to levels of lung function above that conferred by the Gly16 allele, thereby avoiding morbidity and mortality. This hypothesis is supported by data showing a greater bronchodilator response in children homozygous for Arg16 com-

pared with Gly16 treated with a single dose of bronchodilator [11]. Thus, the evidence suggests that the Arg16 allele may be a 'response' allele and the Gly16 a 'severity' allele: Arg16 can result in higher lung function with certain stimuli and lower lung function with other stimuli, whereas Gly16 is not favourable in asthmatics since it does not allow the same degree of control of airway smooth muscle tone and variation in airway calibre.

The association between the genotype homozygous Gly16 and asthma admission may be related to functionality and expression of the β_2 AR that are influenced by the Arg16Gly polymorphism. Children with the genotype homozygous Gly16 have a blunted response to a single treatment with short-acting bronchodilator when compared with the genotype homozygous Arg16 [11]. *In vitro* studies have demonstrated that the Gly16 genotype is associated with a diminished response to β agonist following persistent exposure to bronchodilator medication [25]. This response appears to be related to enhanced down-regulation of the β_2 AR associated with the Gly16 allele and has been reproduced in one *in vivo* study [26] but not others [27, 28]. During an exacerbation of asthma, a child is likely to be treated with a β agonist and individuals homozygous for Gly16 will be less responsive and thus require repeated treatment with bronchodilator that may induce down-regulation of the β_2 AR. This may render a child homozygous for Gly16 relatively insensitive to inhaled β agonist therapy in the event of an asthma exacerbation, thereby prompting hospitalization.

We have previously demonstrated an association between increased AR in infancy and asthma at 6 years of age [1] yet in the current study, we report that increased AR at 1 month was associated with variations of the β_2 AR but not asthma at 6 years of age. From this cohort, we have previously demonstrated an association between viral-induced wheeze, but not asthma, and the genotype homozygous Arg16 [6]. This suggests that there may be a relationship between increased AR in infancy, viral-induced wheeze in childhood and the Arg16 allele but not asthma *per se*, although some cases with recurrent viral-induced wheeze may be labelled as asthma.

There is no universally accepted method for describing AR in infants and in the current study we have used two methods, PC₄₀ and DRS. The genotype homozygous Gly16 was associated with lower AR for both measures of AR although the relationship reached statistical significance for DRS compared with PC₄₀. The DRS and PC₄₀ are different methods for interpreting the same response to histamine and as such both would be expected to have similar associations with the Arg16Gly polymorphism. There are very limited data relating to factors associated with increased AR in infancy and we have therefore chosen to present both PC₄₀ and the DRS association study results.

The data presented in this study suggested that the Arg16Gly polymorphism of the β_2 AR was relevant to AR and lung function throughout childhood, although the difference seen in lung function was almost certainly of no clinical relevance. Clearly, numerous other genetic and environmental factors are also likely to influence this relationship. Our observation that polymorphisms of the β_2 AR were associated with both AR and lung function throughout

childhood, nonetheless, suggests that these polymorphisms are important determinants of these attributes.

In summary, this study demonstrated that the relationship between increased AR in infancy and reduced lung function in childhood might be attributable in part to the Arg16Gly polymorphism. The Arg16Gly polymorphism also appeared to be related to severe asthma but not asthma *per se*. We suggest that in addition to tobacco smoke and intrinsic variations in the airway epithelium [29], reduced airway smooth muscle tone that is genetically determined may also result in both reduced lung function and increased AR.

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